FATE OF NUTRIENTS AND CARBON IN SLUDGE MINIMIZING CANNIBAL™ TYPE OF LABORATORY SCALE REACTORS

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Excess biomass generated during municipal wastewater treatment using biological activated sludge processes is one of the main drawbacks of treatment processes. In this study, two sequencing batch reactors (SBRs) were operated and monitored to evaluate, (1) the long-term sustainability of sludge reduction and nutrient removal and (2) fate of carbon in the lab scale reactors as well as in full scale plants run under similar sludge reduction modes. One of the lab scale reactors (called control SBR) was run in standard operational mode at 10-day solids retention time (SRT) while the other reactor (called modified SBR) was run in sludge minimizing mode at nearly 100-day SRT to induce the anaerobiosis of the returned biomass in a sidestream reactor. Furthermore, to compare the overall biomass yields in both reactors, the wasted biomass from the conventional reactor was taken to a conventional anaerobic digester. Overall, both reactors achieved an average PO_4^{3-}-P removal of 85%, NH_3-N removal of 99%, and 100% chemical oxygen demand (COD) removal. The modified SBR consistently showed a biomass yield of 0.136gVSS/gCOD as compared to the control SBR which maintained a biomass yield of 0.34gVSS/gCOD. Overall, the modified SBR generated 60% less biomass than the control SBR. Carbon mass balance and partitioning experiments based on C13 substrate showed that both modified SBR and the associated sidestream reactor showed better mineralization in terms of CO_2 production. Furthermore, for the modified SBR, less C13 partitioned into biomass and more C13 went into head space in the form of CO_2, thus

suggesting why modified SBR enabled low biomass yield. A similar trend was observed for full scale missed liquor samples.

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CHAPTER 1

INTRODUCTION AND RATIONALE

Activated sludge process is the most widely used treatment method for municipal wastewater (Grady et al., 1999; Metcalf and Eddy, 1994). The process can be optimized for biological removal of nitrogen and phosphorus using different reactor configurations, in addition to the effective removal of organic matter and suspended solids (Grady et al., 1999; Metcalf and Eddy, 1994). Figure 1 shows a typical configuration of activated sludge process bioreactor.

The influent after going through primary treatment (i.e., screening, primary settling) contains various contaminants (organics, nitrogen and phosphorus) of concern and is allowed to enter the bioreactor. These contaminants serve as carbon, nitrogen and energy source for the bacterial community present in the bioreactor and are nearly removed by the time the influent leaves the bioreactor. As a result, bacteria grow and multiply. The treated liquid waste flows to the gravity secondary clarifier where most of the biomass settles down at the bottom of the clarifier. Because wastewater treatment is a continuous process, as shown in Figure 1, the settled biomass in the secondary clarifier is routinely removed from the bottom, a portion of this removed biomass is recycled back to the bioreactor to maintain a healthy population of bacteria in the bioreactor and a major portion is wasted on daily basis. As shown in Figure 2, the wasted biomass needs further processing and is labor, cost and energy intensive.

The treatment of excess sludge is expensive and may account for 25 to 65% of the plant's operation cost (Saby et. al., 2003; Chen et al., 2001 & 2003; Camacho et al., 2002; Cui and Jahng, 2004; Barjenbruch and Kopplow, 2003). Hence, the excess sludge is the one of the main drawbacks of the activated sludge process. Treatment of this excess sludge requires much energy and labor and, excess sludge is a big environmental concern in our present environmental sustainability driven society. Hence, sludge reduction at wastewater treatment plants is increasingly attractive due to rising costs and constraints associated with sludge treatment and disposal.

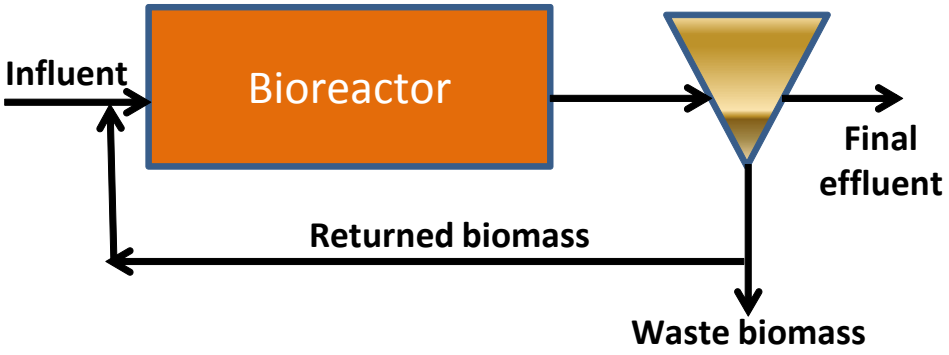
Approximately 8.2 million tons of sludge were generated in 2010 in the United States, and the amount has been predicted to reach over 10 million tons by the year 2012 (USEPA, 1999). Anaerobic digestion reduces the excess biomass by 40~50 % with methane gas being a useful byproduct, albeit a green house gas. Several research efforts have also shown that electricity (Liu et al., 2004, Min and Logan, 2004) and hydrogen gas (Angenent et al., 2004; Hallenbeck, 2005 and Gong et al., 2005) can be generated biologically. However, challenges still exist regarding the improved yields of electricity and hydrogen gas using microbial fuel cell and biomass fermentation techniques, respectively. Another option for the use of biomass includes its composting followed by land application. However, land application of biosolids is restricted in many states due to health risks to people and livestock because of potentially toxic elements in the sewage sludge, i.e., heavy metals, pathogens, and persistent organic pollutants and nutrients (Wei et al., 2003). Handling and disposal of excess sludge is more challenging in coastal areas such as Florida and California and, in coastal countries like Malaysia, Singapore and Indonesia due to depleting landfill resources and other environmental concerns. Hence, it

is highly debatable that excess biomass is a useful commodity (Ødegaard et al., 2002). **As a result, excess biomass from activated sludge processes is regarded as an environmental concern which threatens the sustainability of activated sludge treatment processes.** The reduction in sludge could dramatically impact the difficulties municipalities are facing today in disposing of or reusing their excess sludge.

For sludge reduction at the source, a number of technologies have been developed that are single or a combination of physical, chemical, biological and thermal processes (reviewed by Ødegaard, 2004) (Figure 3). However, cost savings from sludge minimization using one or a combination of physical, chemical, biological and thermal processes must be compared to costs involved in implementing these processes. All these alternatives are expensive and could increase the overall energy consumption of the plant (Böhler and Siegrist, 2006).

Sludge minimization through anaerobiosis (Westgarth, 1963; also called the fasting of biomass) of returned activated sludge using a sidestream anaerobic reactor (Figure 4) is a relatively new sludge minimization approach which has been primarily investigated in laboratory scale set ups with few full scale installations in the U.S. with the trade name of Cannibal^{TM}. As shown in Figure 4, a portion of the returned biomass is taken to an anaerobic sidestream reactor (fasting or anaerobiosis of sludge) and an equal volume of the mixed liquor from this sidestream reactor is sent back to the main bioreactor (feasting conditions). The circulation of biomass through the anaerobic sidestream to the main bioreactor causes a net reduction in the overall observed biomass yield. Figure 4 depicts that one-tenth of the underflow is going through the sidestream and the rest is by passing this sidestream in the form of returned activated sludge. Likewise, one-tenth of the mixed

liquor from the sidestream tank is sent back to the main bioreactor. Cycling of a portion (one-tenth in this case) of the secondary clarifier underflow through the anaerobic sidestream tank induces certain conditions (not known fully) under which the process depicted in Figure 4 achieves a net reduction (up to 60%) in the biomass. Despite many significant advantages, several factors preclude the widespread application of activated sludge configurations which achieve a net sludge reduction through biomass fasting and feasting. These factors include; (1) the absence of information on the fate of carbon, i.e., lack of carbon mass balance, (2) the absence of nutrient removal component in these processes, (3) the lack of proven mechanisms of sludge reduction in these processes and, (4) absence of a well established design and operational strategy. From a wastewater operator's view point, the last two are more essential because nutrient removal is mandated by federal and state regulatory agencies to protect the quality of receiving waters and, the consulting world does not have a sound understanding on the fate of carbon in these sludge minimizing processes.



Typical configuration of a conventional activated sludge process. Biomass wastage needs further processing which is cost and energy intensive

Figure 1: Typical configuration of activated sludge bioreactor

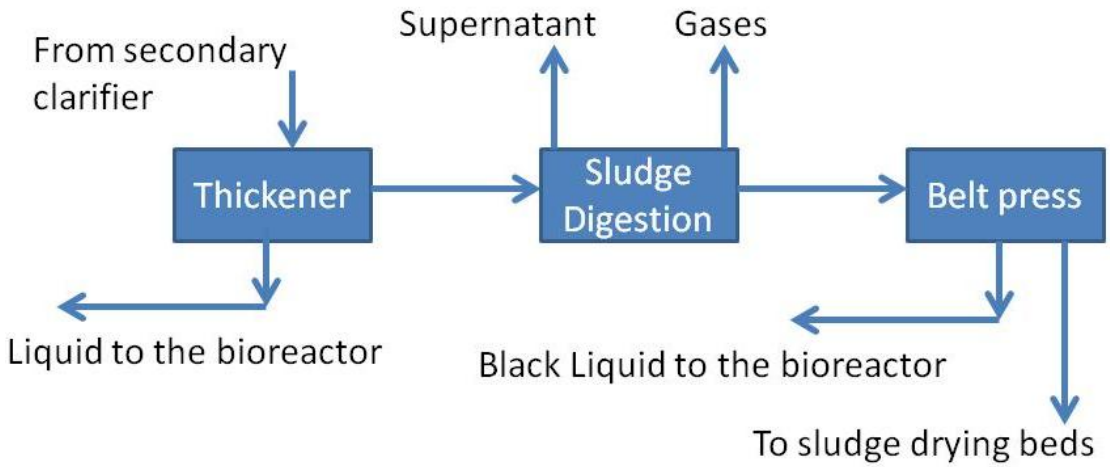


Figure 2: A general flow scheme most commonly used for processing the excess biomass/sludge

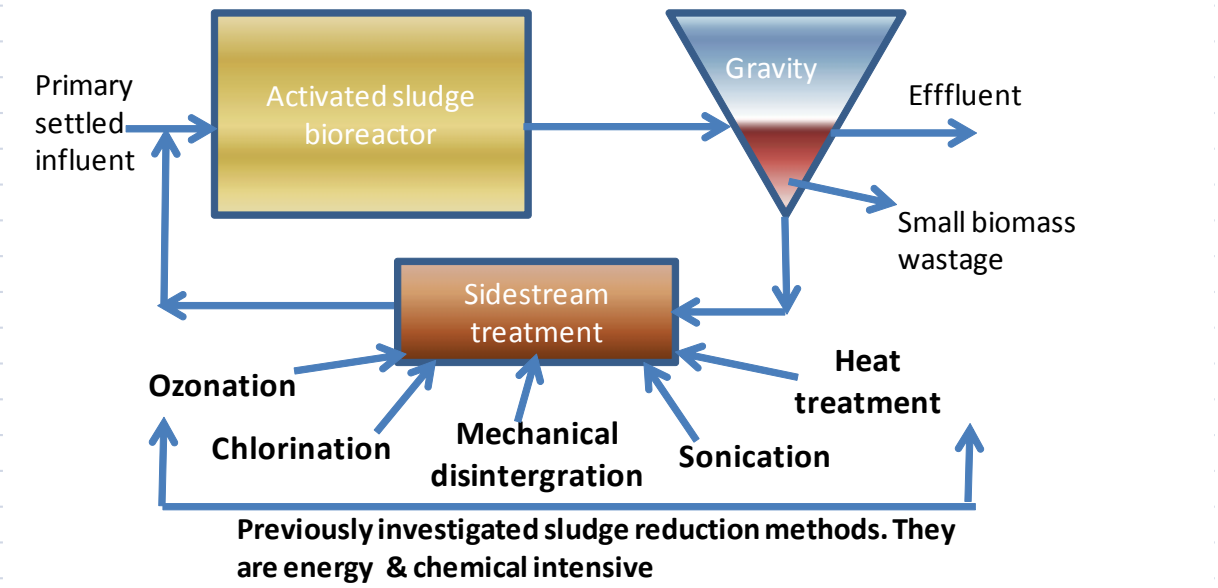


Figure 3: The schematic shows a conventional activated sludge process configuration. Biomass wastage from the conventional configuration is evident

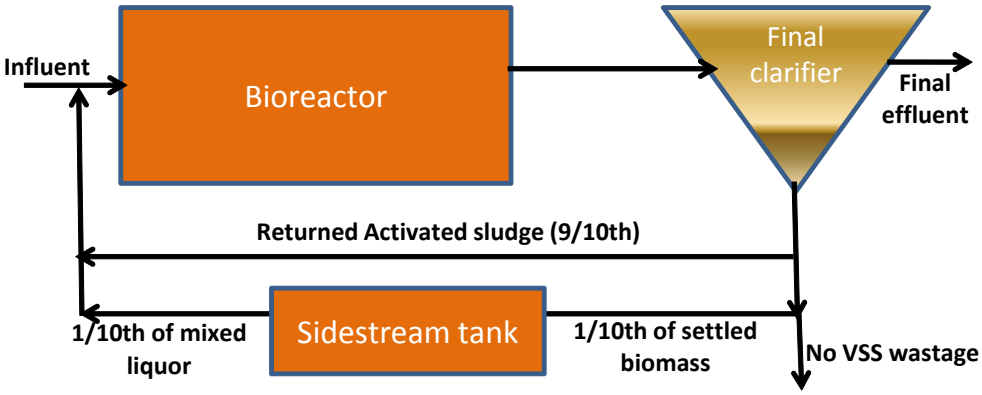


Figure 4: A schematic of a typical sludge minimizing activated sludge process through returned biomass fasting (in the sidestream tank) and feasting (in the bioreactor). Examples include oxic settling anoxic process (primarily lab or pilot scale) and Cannibal^{TM} process (some full scale applications).

CHAPTER 2

RESEARCH OBJECTIVES

As stated before, nutrient removal and understanding the fate of carbon in sludge minimizing bioreactors have been investigated but remain an issue for the successful acceptance of these processes in wastewater industry. Although, issues like mechanisms of sludge reduction, design practices and microbial ecology related to these innovative processes are equally important, I choose to address the research questions related to the fate of carbon and nutrient removal in sludge minimizing processes because of the time frame of my master's thesis.

Main nutrients of concern are nitrogen and phosphorus. Biological nitrogen removal requires nitrification and denitrification processes to be incorporated in the treatment train through the operation of oxic and anoxic zones. It is feasible for an operation to significantly minimize sludge production via feasting and fasting without significantly impacting nitrogen removal if the adequate key microorganisms remain viable. Coupling sludge minimization with biological phosphorus removal through enhanced biological phosphorus removal (EBPR) could be challenging and more complex. Activated sludge processes achieving sludge minimization using anaerobiosis (fasting and feasting) have been operated at nearly infinite or very high solid retention time (SRT). This becomes a challenge, especially for EBPR, because the successful operation of EBPR requires the process to be operated at a finite SRT, typically at 5-15 days (Rodrigo et al., 1996; Shao et al., 1992; Fukase et al., 1985)

Hence, I posed the following two questions.

1. Can biological nitrogen (N) and phosphorus (P) removal be sustained in sludge minimizing reactor and if yes, at what biomass yield?
2. What is the fate of carbon in a sludge minimizing reactor?

To answer these questions, I ran two laboratory scale sequencing batch reactors, one in the standard operational mode and the other in sludge minimization mode. Both reactors were operated to achieve nitrogen and phosphorus removals. I used stable isotope of carbon to accomplish carbon mass balance in these reactors. Furthermore, there are several full scale sludge minimizing plants in the U.S. and around the world which are running the scheme (sludge minimization mode) shown in Figure 4 under the trade name of Cannibal^{TM} marketed by SIEMENS Water Co. Hence, my carbon mass analysis also included sampling several full scale Cannibal plants. The specific objectives of this research were:

1. Monitor the performance of two laboratory scale SBRs in terms of nutrient removal and biomass yield.
2. Calculate oxygen uptake rates (OURs) in both SBRs.
3. Conduct carbon mass balance in these two SBRs using C13 carbon substrate.
4. Conduct carbon mass balance in mixed liquor received from several full scale Cannibal plants.

CHAPTER 3

MATERIALS AND METHODS

Reactor Operation

Two, 2 L bench-scale SBRs were operated to achieve simultaneous ammonia and phosphorous removal along with sludge reduction. The overall schematics of these two systems are depicted in Figure 5. One reactor (called the control SBR) was run in standard operation at 10-day solid retention time (SRT) and the second reactor (called the modified SBR) was run in sludge minimization. Briefly, to induce fasting and feasting of the returned biomass, one-tenth of the settled biomass from the modified SBR at the end of each cycle was brought to a sidestream reactor. Following this, one-tenth of the mixed liquor from this sidestream was recycled back to the modified SBR at the beginning of each cycle.

Recycling of one-tenth of biomass back and forth enabled an overall internal SRT of 10-day in the modified SBR. Furthermore, the modified reactor was run at "small biomass wastage" rate rather than at infinite SRT to sustain efficient EBPR and to avoid any biomass build up in the reactor system. On the other hand, one tenth of the settled biomass from the control SBR was taken to a conventional anaerobic digester operated at 10-day hydraulic retention time (HRT)/solid retention time (SRT) and the observed biomass yield in the control SBR was calculated after the biomass was digested in the conventional digester.

The cycle of each SBR consisted of 5.5 h of reaction period, the distribution of which is shown in Figure 6. At the end of each cycle, 670 mL of supernatant was at the beginning of the next cycle. With a 2 L capacity in each SBR, this corresponded to a hydraulic retention time (HRT) of 18 h. Anaerobic conditions were maintained by constantly bubbling the mixed liquor with nitrogen gas at a rate of 1 L min^{-1} during the specified anaerobic and anoxic time periods of each cycle. Anaerobic conditions were verified by measuring dissolved oxygen in the mixed liquor periodically. I used an automated pH controller to maintain the pH around 7.5.

Reactor performance was monitored in terms of dissolved COD, phosphorus (P) and ammonia removals and the biomass yield was calculated based on total suspended solids (TSS)/volatile suspended solids (VSS) concentrations measured in SBRs, digester and sidestream and effluents. Observed yield was determined by Metcalf and Eddy (2004), which was the ratio of the amount of biomass produced to the amount of substrate consumed. In this study, the observed yield was determined over a given range of operation as the VSS increase/COD used, using all the data over the range of operation for which the yield was calculated. The cumulative wastage was calculated from sampling wastage, effluent wastage and average biomass growth.

Oxygen Uptake Rate (OUR)

Specific oxygen uptake rate (SOUR) describes the amount of oxygen used by the microorganisms to consume 1g of food and is reported as mg/l of oxygen used per g of organic material per hour, and is used to monitor performance of process in biological wastewater treatment facilities. After reactors became steady state, 6 SOUR tests, which included spiked and unspiked SOUR tests, were performed in this study to estimate

organism response/physiology in both reactors. The unspiked SOUR was measured at the end of the cycle due to the endogenous respiration of the activated sludge. The spiked SOUR tests were added a known substrate to the unspiked samples to predict how the organisms in the mixed liquor respond to that substrate. In this study, first spiking with feed A, which can estimate heterotrophic activity; second spiking with feed B, the OUR response will estimate only nitrification activity in a mixed liquor sample. The SOUR was measured using method 2710B from the "Standard Method for Examination of Water and Wastewater." The oxygen uptake rate was measured using a dissolved oxygen meter.

Carbon Mass Balance in Twin Reactors. For carbon mass balance experiments using C13 labeled acetate as carbon substrate, 70-mL glass serum bottles were used. For experiments with mixed liquor from the main SBRs, biomass was collected at the end of a specific cycle and then diluted with distilled water (DI). For the digester associated with the control SBR, and sidestream reactor associated with the modified SBR samples, biomass samples were collected and mixed with biomass samples in a 1:10 ratio to simulate the interchange rate. For both sets of experiments, biomass samples were transferred to a 70-mL serum vial, which was immediately capped with an air tight Teflon cap and an aluminum crimp. The contents in the vial were spiked with a stock solution of nutrients and C13 labeled carbon substrate for main SBR samples, and with preacclimatized C13 labeled biomass (in 1:10 ratio) for digester and biomass samples. For samples from the main reactors experiments were conducted under aerobic conditions, and for sidestream and digester samples experiments were conducted under anaerobic conditions. Figures 7 and 8 show the overall scheme for the aforementioned

serum bottle tests for the control SBR system and the modified SBR system, respectively.

Carbon Mass Balance in Mixed Liquor from Full Scale Plants

A total of 10 full scale Cannibal^{TM} plants were analyzed for carbon partitioning between the biomass and the head space gases. The strategy which was used for biomass from the lab scale reactors was used for full scale experiments except that the acclimatized C13 biomass was not used for serum bottle tests on mixed liquor from sidestream reactors. Instead, C13 glucose was used to spike the mixed liquor. This strategy was employed because it was not possible to acclimatize the biomass with C13given the short duration of the shipment of mixed liquor samples from different plants. The details of the plants are provided in Table 1. For all plants, glucose with only one C-atom C13 labeled was used. Figure 9 provides the locations of all 10 full scale plants in the U.S.

Head Space Gas Analysis

All analysis related to C13-CO_2 in the head space was done at the isotope core facility. A known volume of head space from the serum bottle was injected into the injector port. The final outcome from the gas chromatograph was C12/C13 ratio which was used to calculate the mass of C13 in the head space. Ideal gas laws were used to calculate the moles of gas in the head space. With the total moles of gases and C13/C12 ratio, it was easy to calculate the mass of C13 in the head space.

C13 Analysis in the Biomass

A known volume of biomass was oven dried overnight at 103^{o}C. The dried biomass was crushed to homogenize and then a known weight was ignited in a controlled confined

space connected to the Gas Chromatography (GC) port. The rest of the analysis protocol was similar to what was used for the head space gases. Figure 10 shows the general schematic about how head space and biomass samples were analyzed and how the resulting information was used to calculate molar concentrations of C13.

Calculation for Carbon Mass Balance

The following section depicts protocol that was used for carbon mass balance. In the headspace, the unknown CO_2 percentage was determined by comparing with the known standard gas (equation [1]). The CO_2 concentration as mol was obtained using ideal gas law (PV=nRT).

$$percentage\ (unknown) = \frac{area\ (unknown) \times percentage\ (std)}{area\ (std)} \quad [1]$$

To get the C13 concentration using equation [2], another data called atom percentage (AT%C13) was used, which was obtained from the core facility for each sample analyzed.

$$n(\ \ C13) = n(CO_2) \times AT\%\ \ C13 \quad [2]$$

Specific CO_2 and C13 production was determined by CO_2 or C13 produced and VSS (as equation [3]). Percentage recovery of C13 in headspace or solids was calculated by equation [4].

$$Specific\ production\ (CO_2\ or\ \ C13) = \frac{n(produced)}{gVSS} \quad [3]$$

$$percentage\ recovery\ (\ \ C13\ in\ headspace\ \ or\ in\ solids) = \frac{C13\ (produced)}{C13\ spiked} \times \% \quad [4]$$

The total C13 recovery, which used for the analysis of lab-scale mainstream and all full-scale scales carbon mass balance, includes C13 recovery in headspace and in the

solids (equation [5]). Equation [6] used to calculate the C13 recovery in sidestream biomass of the lab scale reactors.

$$\mathrm{C13(recovery,\%)} = \text{percentage recovery (}\ \mathrm{C13\ in\ headspace}) + \text{percentage recovery (}\ \mathrm{C13\ in\ solids}) \quad [5]$$

$$\mathrm{C13(recovery,\%)} = \frac{\Delta\mathrm{n}(\ \mathrm{C13})\ \text{in the headspace}_*}{\Delta\mathrm{n}(\ \mathrm{C13})\ \text{in the solids}} \quad [6]$$

Calculation of CO2 Production inside the Serum Bottle

Based on the ideal gas law, the total moles of gas in the headspace can be calculated using equation [1] (assume the pressure and temperature inside the bottle were the same, 1atm=101325Pa, 25^{o}C=298K). The moles of CO_2 will be determined by equation [2], using the result from equation [1] times the CO_2 percentage.

$$n(gas) = PV/RT \quad [7]$$

$$n(CO_2) = n(gas) \times CO_2(\%) \quad [8]$$

Gas Chromatographic Conditions

Using a gas tight syringe, a 0.4 mL sample was obtained from a headspace vial and injected into a modified Elemental Analyzer (model 1110, Carla Erba, Milan, Italy). The sample passed through a gas chromatography column (Poraplot Q©, 3 m length, 80°C) and entered a Delta Plus isotope ratio mass spectrometer (Finnigan-MAT, Bremen Germany) via an open spit interface. Stable isotope ratios for laboratory reference materials were calibrated using NBS-19 for C. The standard deviations (SD) of repeated measurements of the same commercially produced powdered keratin reference material throughout all protein analyses were 0.2 for C. Triple 200ul standard gas was injected for the purpose of calibration. From the area of the peak of CO_2, the percentage of the

unknown sample can be calculated based on the standard calibration sample, and thus the concentration of the sample could be calculated.

Analytical Methods

Samples were routinely collected at the end of each period, filtered (0.45 m)and analyzed. Chemical oxygen demand (COD), ammonia (NH_3-N), nitrate (NO_3-N), nitrite (NO_2-N), and dissolved phosphorus (PO_4^{3-}P), were quantified using HACH methods 8000, 10031 (Salicylate method), 10020 (Chromotropic acid method), and 8153 (Ferrous sulfate method), and 8048 (Ascorbic acid method), respectively. Mixed liquor samples were collected using plastic pipette, which was put into midheight on the bioreactors, effluent cowboy or holding tank. The mixed liquor solids concentration was determined as total suspended solids (TSS) and as volatile suspended solids (VSS), both were measured in accordance with standard methods (APHA, 1985). Sludge volume index (SVI) was determined by using method 2710D from “standard method for examination of water and wastewater.” (APHA, 1985)

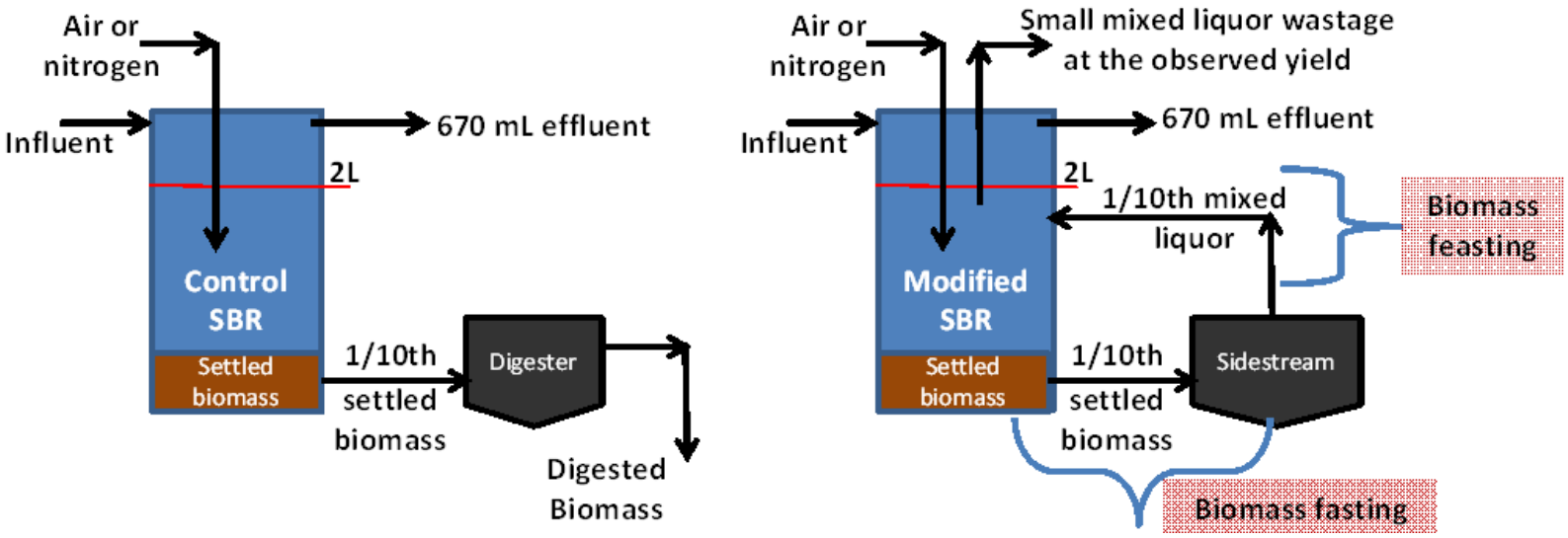


Figure 5: Schematics of the control (left side) and the modified (right side) SBRs

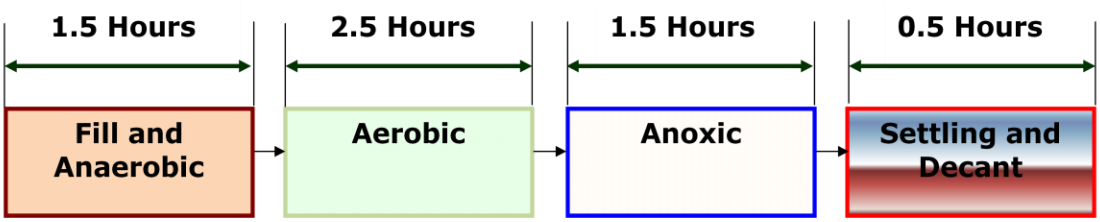


Figure 6.Time sequence in each cycle of the control SBR and the modified SBR. The anaerobic phase is provided to release P, the following aerobic will enable P uptake and ammonia oxidation and the last anoxic time period is provided to allow any denitrifictaion.

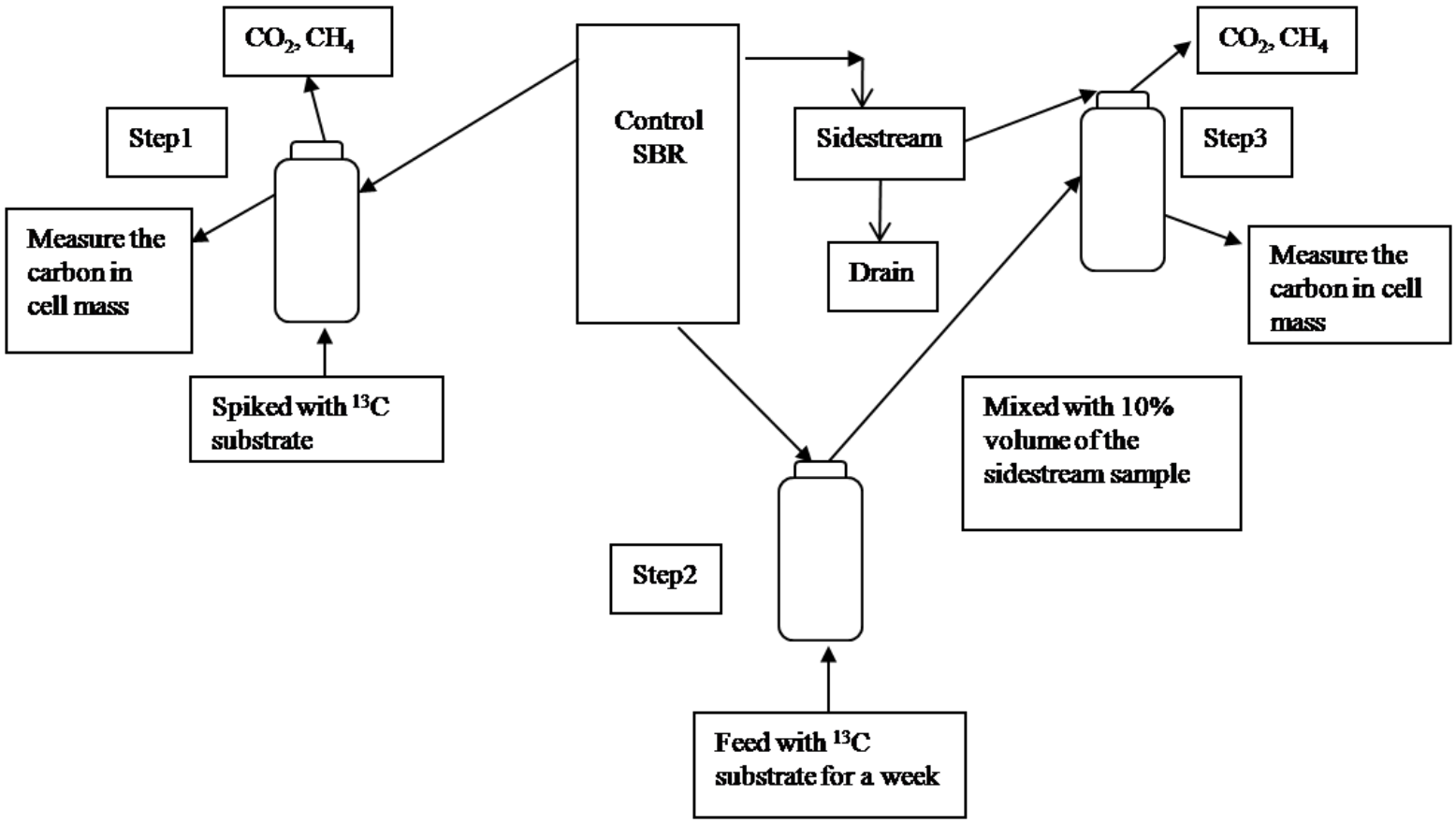


Figure 7: Serum bottle carbon mass balance scheme for the control SBR system

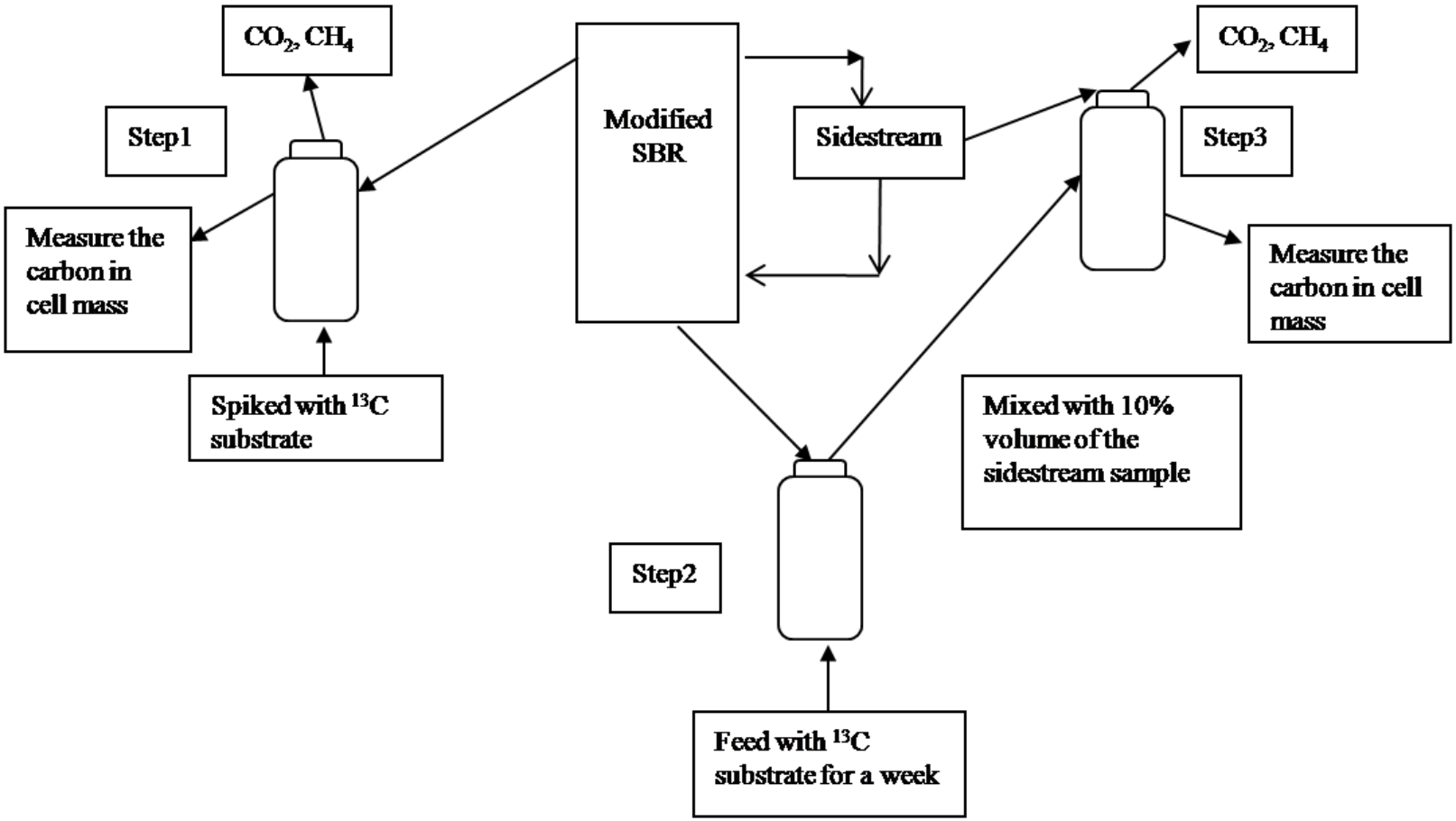


Figure 8: Serum bottle carbon mass balance scheme for the modified SBR system

Table 1: Treatment plants investigated

Plant Name and location	Treatment system	Pre-Cannibal Yield (lbTSS/lbBOD)	Post-Cannibal Yield (lbTSS/lbBOD)
Peru, IN	8.0MGD VLR	0.8	0.1
Lebanon, OR	3.0MGD CAS		
Albany, OR	16.0MGD VLR		
Clovis, CA	2.8MGD MBR		
Emporia, VA	1.5MGD Oxidation Ditch	0.6	0.35
Morongo, CA	0.75MGD SBR	unknown	0.1-0.2
Big Bear, CA	3.5MGD Oxidation Ditch	1.0	0.8
New Miami, OH	1.0MGD VLR		
So. Ft. Collins, CO	4.5MGD VLR	0.75	0.65
Central Valley, UT	SCB		NA

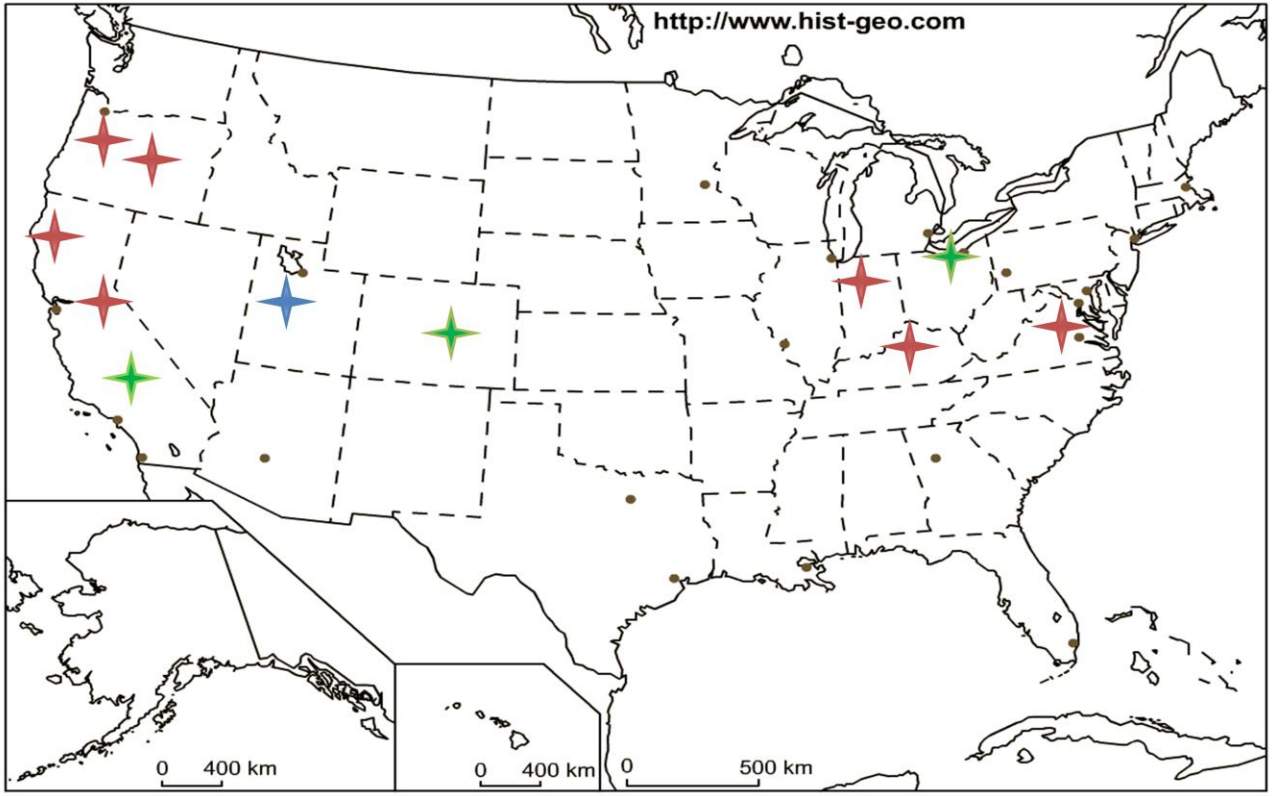


Figure 9: Map showing the 10 wastewater treatment plants' locations (www.hist-geo.com)

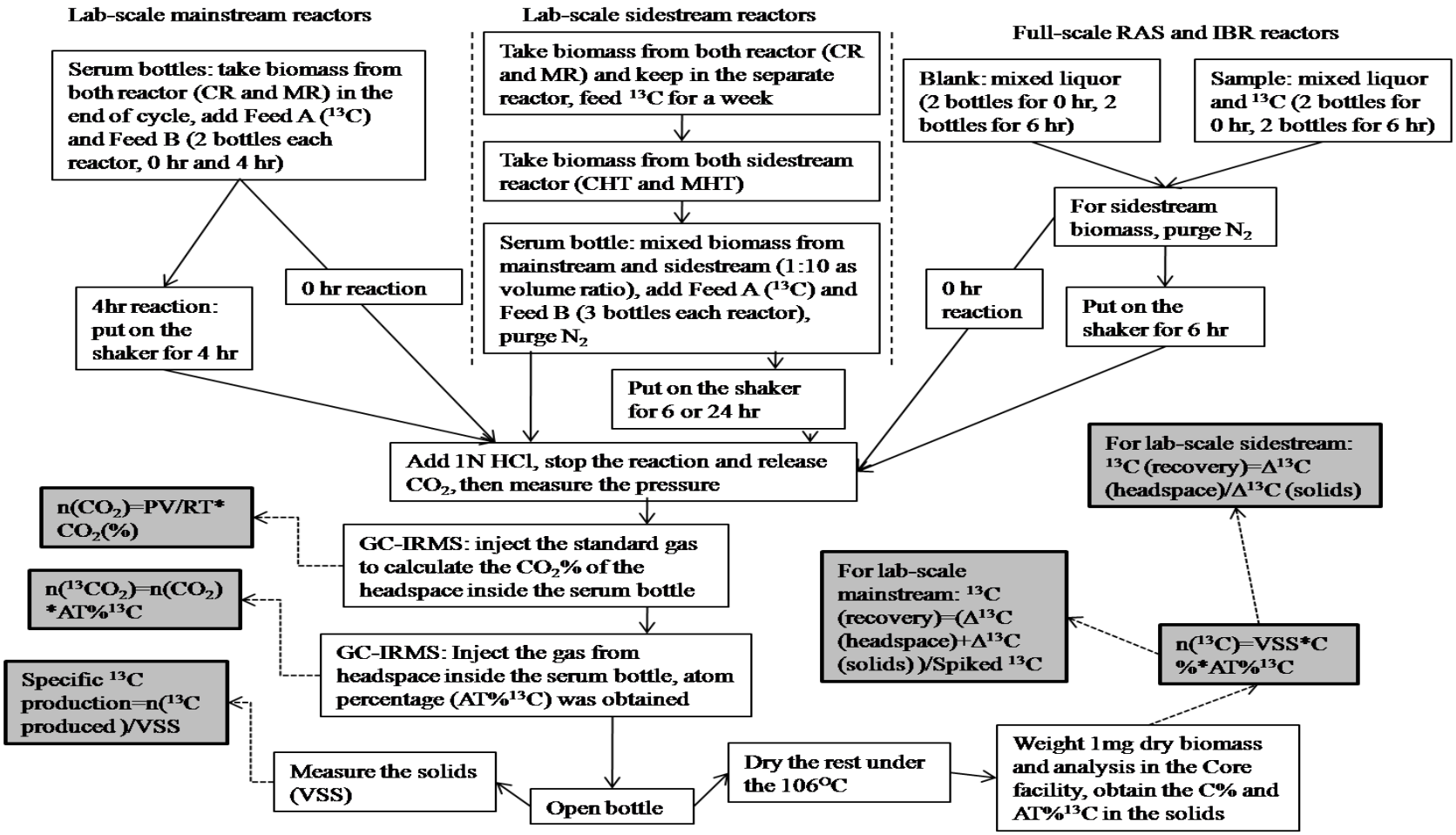


Figure 10: General Schematic for C13 analysis and calculation. In this schematic, CR stands for control SBR, MR stands for modified SBR, CHT stands for control anaerobic digester and MHT stands for sidestream reactor associated with the modified SBR.

CHAPTER 4

RESULTS AND DISCUSSION

Twin Reactor Performance

Nutrient Removal. The twin reactors were started almost three years ago but the reactors were restarted in August 2011 because these twin reactors were not operated properly in summer 2011. All tubing, a few pumps, pH controller and SBRs were changed in August 2011 and that marked the beginning of my master's thesis research. At that time, I also prepared a fresh stock solution of micro nutrients and tried to revamp the reactors, which provided me with the experience of reactor start-up and operation. Both reactors were showing poor removal efficiencies. It took almost 2 weeks for me to recover the performance of the reactors.

Figure 11 shows reactor performance in terms of phosphorus removal. This figure shows dissolved P profiles for the influent, effluent and total P release during the anaerobic phase for the control SBR and the modified SBR. The average dissolved P released at the end of the anaerobic phase was about 13.82 mg/Lin the control SBR and 17.65mg/L in the modified SBR, respectively. The dissolved P in the final effluent was always below 1 mg/L in both SBRs. Overall, both SBRs consistently showed 85% or more P removal efficiency. Most of the COD was consumed by the end of anaerobic phase in both SBRs and nearly 100% COD removal was consistently recorded in both SBRs.

Figure 12 shows reactor performance in terms of ammonia removal. It is evident from this figure that the ammonia nitrogen concentration in the final effluent of both SBRs was below detection limit, thus giving nearly 100% ammonia oxidation. Ideally, the ammonia concentration in the influent and at the end of anaerobic phase should be identical but the decreased ammonia nitrogen concentration at the end of anaerobic phase shows one-third dilution effect.

Figure 13 shows NO_2-N concentrations in both SBRs. It is interesting to note that the higher NO_3 concentration (Figure 14) was accompanied by a corresponding decrease in NO_2-N concentrations indicating that more nitrite oxidized to nitrate through the second step of nitrification in the modified SBR. Also, NO_2-N concentrations in the modified SBR were more consistent and stable than in the control SBR. At the end of aerobic period, NO_2-N was occasionally found in both reactors, which indicated incomplete nitrification. At the end of aerobic phase, the NO_2 concentration in the control and modified SBR were 1.63 and 0.25 mg/L, respectively. Also, the difference in NO_3 concentration at the beginning and at the end of anoxic indicated active denitrification in both SBRs. The effluent NO_2-N concentration from the control SBR and modified SBR were 0.85mg/Land 0.03mg/L. As mentioned previously, COD was consumed during the anaerobic phase, denitrification, which required organic carbon source, was not expected during the anoxic phase. The appearance of partial-denitrification during the anoxic period agrees with a previous study by Datta et al. (2009). This could probably be attributed to the denitrifying polyphosphate-accumulating organisms (DNPAOs) (Saito et al., 2004). DNPAO that use stored polyhydroxyalkanoates (PHA) as a carbon source for

denitrification, recorded and also exhibited uses cyclic phosphate release and uptake when exposed to a sequence of anaerobic and aerobic conditions.

Solids and Biomass Yield

Figure 15 shows TSS and VSS in the control SBR and the modified SBR. The average TSS and VSS concentrations in the control SBR were 3342 and 2984 mg/L, respectively. Furthermore, VSS followed the same trend as that of TSS in the control system. In the modified SBR, the solids built up from day-113, which was because the settling characteristic of the modified SBR had changed. On the other hand, the solids performance in the anaerobic chemostat tank (CHT) associated with the control SBR was 3419mg/L and 2521 mg/L for TSS and VSS, respectively (Figure 16).

The tubing was not long enough to reach to the settled biomass during the settling period and supernatant was pumped into the sludge holding tank and the diluted sludge was returned back to the SBR attend, therefore, the sludge concentration in the holding tank decreased. Following manual wasting of biomass from day 120 to 154, TSS and VSS concentrations in the modified SBR decreased from 5110 to 3665 mg/L and from 3600 to 2500 mg/L, respectively. After the 154th day, the modified SBR reached a steady state phase, which can be described as a phase during which the solids showed minimum variation. During the steady state, the VSS in the modified SBR remained constant, which means the solids stopped building up in this period, but not the TSS. The fluctuations in the TSS values could be because of the absence or presence of particulate inorganics in the mixed liquor (Datta et al., 2009). In the solids profile in the sludge holding tank attached to the modified SBR, average TSS and VSS concentrations were 3926 and 2726 mg/L. By the 113th day, TSS decreased from 3580mg/L to 2920mg/L, and

started to increase after the 122nd day. This fluctuation was because of manual wasting which avoided solids going back in the main reactor. After the steady state, the solids concentration in the anaerobic chemostat tank (MHT) associated with the modified SBR remained constant.

Based on the solids data and the mass of COD that was fed to each SBR, the biomass yield was calculated to be 0.333 and 0.114 mg VSS/mg COD for the control SBR and modified systems, respectively. The yield in the modified SBR was 60% less than the control SBR. According to an earlier study by Novak et al. (2006) on the Cannibal process, the yield in the control system was around 0.26mg VSS/mg COD, which was slightly different from this study (0.333mg VSS/mg COD). The reason for the difference could be attributed to the SRT of both systems. The SBR was operated with an SRT around 20 days, as compared to 10 days in this study.

Figure 17 shows linear regression that was performed on cumulative solids and COD plot to calculate biomass yields. The foretasted biomass yields for the control and the modified SBR systems are obvious in the plot. This graph also shows a third data fit line (inverted gray triangles). This third line is the regression fit for the cumulative solids and COD for the control SBR reactor and does not account for solid digestion in the conventional anaerobic digestion associated with the control SBR. Recall, the previously reported biomass yield (i.e., 0.333) for the control SBR system accounted for solid's digestion in the conventional anaerobic digester.

As Figure 17 shows, the observed biomass yield in the control SBR if the anaerobic digestion is not considered, is 0.651 mg VSS/mg COD as compared to 0.333 mg VSS/mg COD. If we assume that the biomass yield in the modified SBR would have also been

0.651 if the associated sidestream is not considered, the difference (from 0.651 to 0.136) in the observed yield is almost 79% when the sidestream is not considered versus when it is accounted for. On the other hand, the corresponding difference in the control SBR is only 49%, which indicated that there are other mechanisms other than just conventional anaerobiosis in the modified SBR system going on to give lower overall yield.

Specific Oxygen Uptake Rates (SOUR) Tests

The SOUR tests aimed to investigate the difference in bacterial activities between both reactors. When both reactors were at steady state, the oxygen uptake rate (OUR) tests were performed by spiking a known volume of mixed liquor from each SBR with feed A (carbon source) and feed B (ammonia) to estimate organism response/physiology. The results of SOUR tests performed under different conditions are shown in Figure 18. Table 2 summarizes the results in form of mathematical values.

When the SOUR tests were done on starving biomass (no spiking with either feed solution), the SOUR values for the MR and CR mixed liquor were nearly the same (top two lines in Figure 18). When biomasses were spiked with either feed A or feed B, mixed liquor from the modified SBR showed higher SOUR rates (Table 2) suggesting the tendency of this mixed liquor to consume dissolved oxygen at a faster rate.

Sludge Volume Index (SVI)

The SVI is considered an important parameter in wastewater treatment to quantify the settling characteristics of activated sludge (Metcalf and Eddy, 2004). The modified system showed good settling properties during the steady state period (SVI: 72 ml/g) compared to the control system (SVI: 136 ml/g). This finding agreed with SVI in lab scale set ups for similar studies (Novak et al., 2007). Lower SVI in modified SBR

indicated much better settling characteristics than in control SBR. The settling result of modified SBR suggested that extracellular polymeric substance (EPS) was regenerated in the SBR, because the flocculation was not negatively affected. Furthermore, the biomass from the modified SBR showed faster settling velocity than the mixed liquor from the control SBR (Figure 19). The effluent characteristic is another parameter to describe the settling properties. The average TSS in the control and the modified SBRs were 64 and 63 mg/L.

Carbon Mass Balance

Fate of Carbon in Control SBR and Modified SBR Using C12. Table 3 summarizes CO_2 formation rate results with mixed liquor taken from the control and the modified SBRs. Based on the ideal gas laws and biomass concentrations in serum bottles, the specific molar concentrations of CO_2 were calculated. It is clear from Table 3 that the mixed liquor from the modified SBR enabled almost 44% more CO_2 than the mixed liquor from the control SBR.

Table 4 shows CO_2 formation results in mixed liquors taken from the control and the modified SBRs. This experiment was repeated with C12 to confirm results shown in Table 3. It is convincingly clear that the results are reproducible and that the modified SBR produced 45% more CO_2 gas than the mixed liquor from the control SBR. Based on these experiments, it can be concluded that the modified SBR mixed liquor poses greater metabolic activity and that this SBR has the capability of mineralizing the organics to CO_2 at a faster rate than the control SBR thus producing less biomass.

Fate of Carbon in Control and Modified SBR Using C13 Isotope and Carbon Mass Balance in The SBRs Based on Spiked C13 Concentrations. An attempt was made to

achieve carbon mass balance in serum bottles in which case, C13 partitioning as a result of spiking in the biomass was also measured. Table 5 shows three subtables. The top of Table 5 represents C13-CO_2 in the head space of serum bottles at 0 time (beginning of the experiments) for both SBRs. The middle portion of Table 5 shows molar C13-CO_2 concentrations in the head spaces after 6 h from the beginning in duplicates. Finally, the bottom portion shows an increase in C13 content in biomasses as a result of C13 spiking. All three measurements were performed simultaneously in same serum bottles.

The first observation from Table 5 is that almost 41% of the spiked C13 mass went to form biomass in case of mixed liquor from the control SBR where as it was about 29.5 % for the modified SBR mixed liquor. This further supports the notion that less biomass in the modified SBR was generated as discussed previously in this thesis leading to low biomass yield. Also, nearly 100 % C13 recovery was achieved in both SBRs.

Carbon Mass Balance in The Digester and The Sidestream. Table 6 shows total CO_2 generated in the head space at 6 h and 24 h when the biomasses from the conventional digester and the sidestream were spiked with C13 labeled biomass (one-tenth). It is clear from the table that both CHT and MHT mixed liquors enabled almost identical amounts of CO_2 after 6 h. Because fresh C13 labeled biomass was used for these experiments, it could be possible that the bacteria present in CHT and MHT mixed liquors were acclimatizing to this new biomass. Nevertheless, both mixed liquors carried out the digestion of the added biomass. Surprisingly, the mixed liquor from the MHT (sidestream) enabled almost 27% more CO_2 gas in the head space after 24 h than the mixed liquor from the control digester. This adds to the notion that not only are the conditions in the modified SBR well suited for faster metabolism, the conditions in the sidestream reactor

also add to better biomass disappearance. Samples were not spiked with C13 glucose. It is interesting to note that C13 carbon was present in the original mixed liquor samples. The presence of C13 carbon in mixed liquor samples is not surprising because these samples came from full scale treatment plants. Based on nonspiked samples (blank) and C13-CO_2 measurements, it appears that the Peru treatment plant yielded the maximum CO_2. On the other hand, based on C13spiked carbon, the Albany and Lebanon treatment plants mixed liquors produced more CO_2 than other plants. Among all plants, it appears that the New Miami treatment plant yielded the least amount of CO_2, both in the blank and in the spiked serum bottles. However, these results need to be verified further using a modified protocol. For example, washing the biomass samples first before conducting the actual samples.

C13 Partitioning into Biomass for Aerated Bioreactor Samples. Table 7 shows C13 partitioning into biomass. These biomass samples were collected from the same serum bottles/mixed liquor that were used to generate information in Table 1.

In Table 7, "Dec" means decrease in C13 concentration in the biomass from the blank serum bottles over 6 h because these tests were not spiked with C13 carbon. On the other hand, "Incr" implies an increase in C13 content in biomass taken from C13 spiked serum bottles. Based on blank tests, the Peru biomass had the maximum decrease in C13 showing a greater tendency to undergo endogenous decay. This observation is in agreement with more CO_2 formation data in the blank test (Table 8) for the Peruvian mixed liquor. Likewise, the C13 content in biomasses for Morongo, Clovis and Murray decreased in the blank experiments. New Miami enabled the least decrease in C13, which is also in agreement with almost no CO_2 formation (Table 8) in New Miami's blank test.

For the biomass which came from spiked samples, the change in C13 was calculated assuming that cells will grow and hence, the final concentration of C13 in the biomass in spiked samples will be more than the concentration at the beginning of each experiment. Negative value in the last column means that the C13 in the biomass decreased over 6 h to start with Peru, the change in C13 in the spiked mixed liquor biomass was negative, suggesting that the C13 content in the biomass decreased over 6 h. However, it should be noted that the net decrease in the spiked samples accounts for the decrease in blank tests as well. Hence, it is absolutely difficult to conclude that none of the available C13-glucose in the bulk liquid partitioned into the biomass. It is also true for Peru that not much CO_2 formed in the spiked serum bottle and as a result, it is highly possible that not much C13 partitioned into the biomass. The maximum increase in C13 in spiked biomass samples was noticed for Emporia, Big Bear and the plant with ID 2963. The CO2 formation rates for these three plants in spiked tests (Table 8) were also in the same range.

C13 Partitioning into Gas for Sidestream Reactor Samples. For mixed liquor samples from sidestream reactors, we could not analyze samples from Murray, Big Bear, New Miami and ID 2963 because the refrigerator in which these were stored got over cooled to negative temperature due to compressor problems and apparently all bacteria were dead. It is also to be noted that all sidestream samples were stored at least for 1 night at 4^oC before they were processed, whereas aerated bioreactor samples were processed immediately upon their receipt.

According to the Table 9, it looks like there was not much CO_2 formed in the head space of the unspiked blank experiments. Likewise, there was not much CO_2 formation in

the head space in spiked experiments except for the Lebanon and Emporia plants. It is worth mentioning that these experiments were performed by spiking the sidestream mixed liquors with a known concentration of C13 glucose. However, this does not reflect reality because, sidestream reactors receive settled biomass from the aerated bioreactors not any soluble substrate. If we did similar experiments for mixed liquor from the ongoing Cannibal reactor, we will need to use C13 labeled biomass rather than soluble substrate to obtain a true picture. Furthermore, these samples should also be processed immediately without letting them sit overnight.

Table 10 shows partitioning of C13 into biomass for mixed liquor samples from sidestream reactors. A negative "Dec" means C13 initially present in the biomass increased over 6 h, which was the case for Peru and Lebanon. This implies that bacteria grew by consuming C13, which was originally present, from the bulk liquid. Likewise, for the spiked samples, "Incr" is obtained by subtracting 6 h C13 concentrations from those initially (0 h) present. Hence, a positive "Incr" means bacteria consumed C13 from the soluble substrate and grew, which is true for all plants but Albany and Emporia.

In summary, a definite trend could not be established for sidestream samples because: (1) they were spiked with soluble substrate rather than C13 biomass and, (2) samples were not processed immediately because of issues related to the number of samples that can processed at any given point in time.

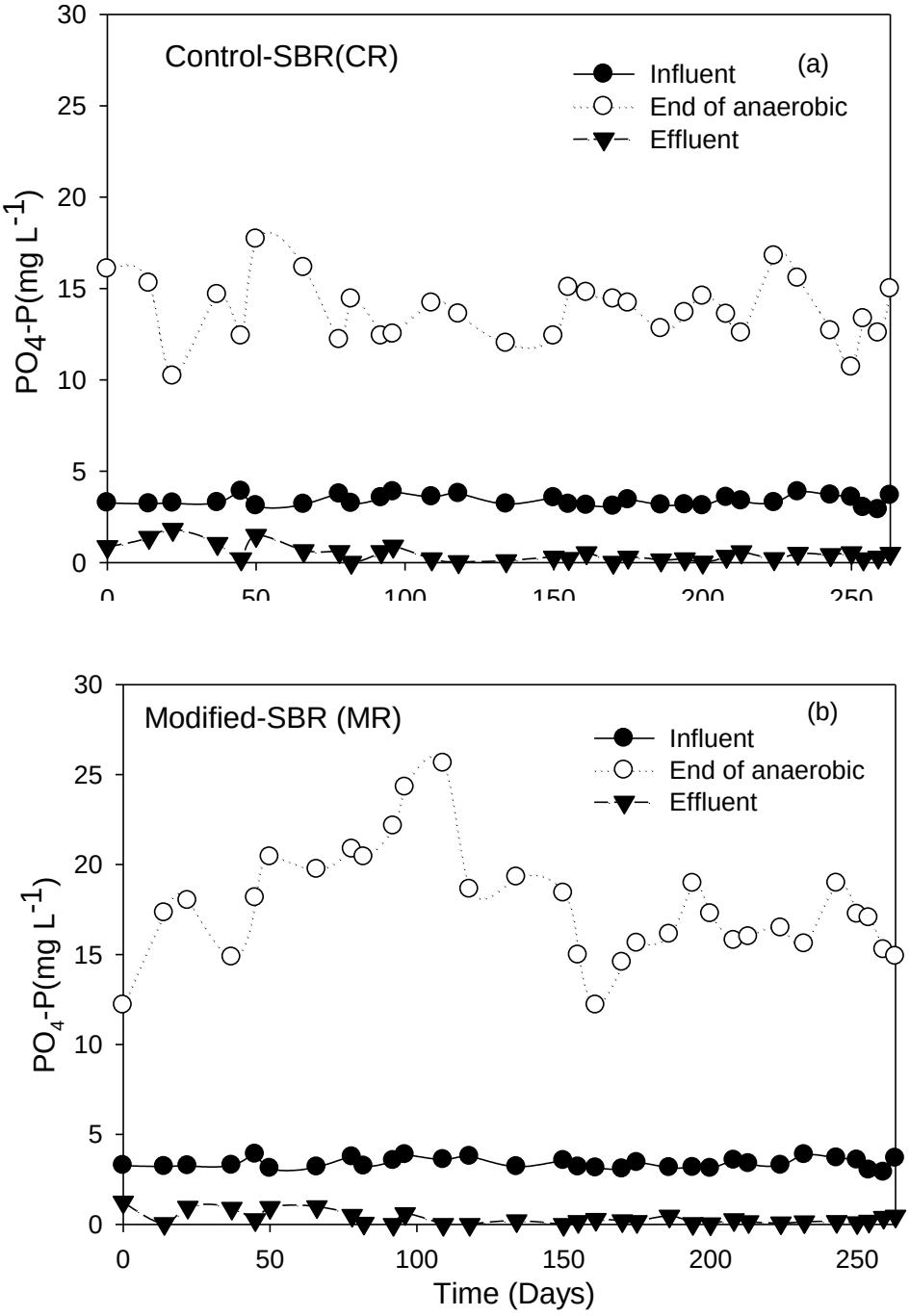


Figure 11: Dissolved phosphorus for the control SBR and the modified SBR

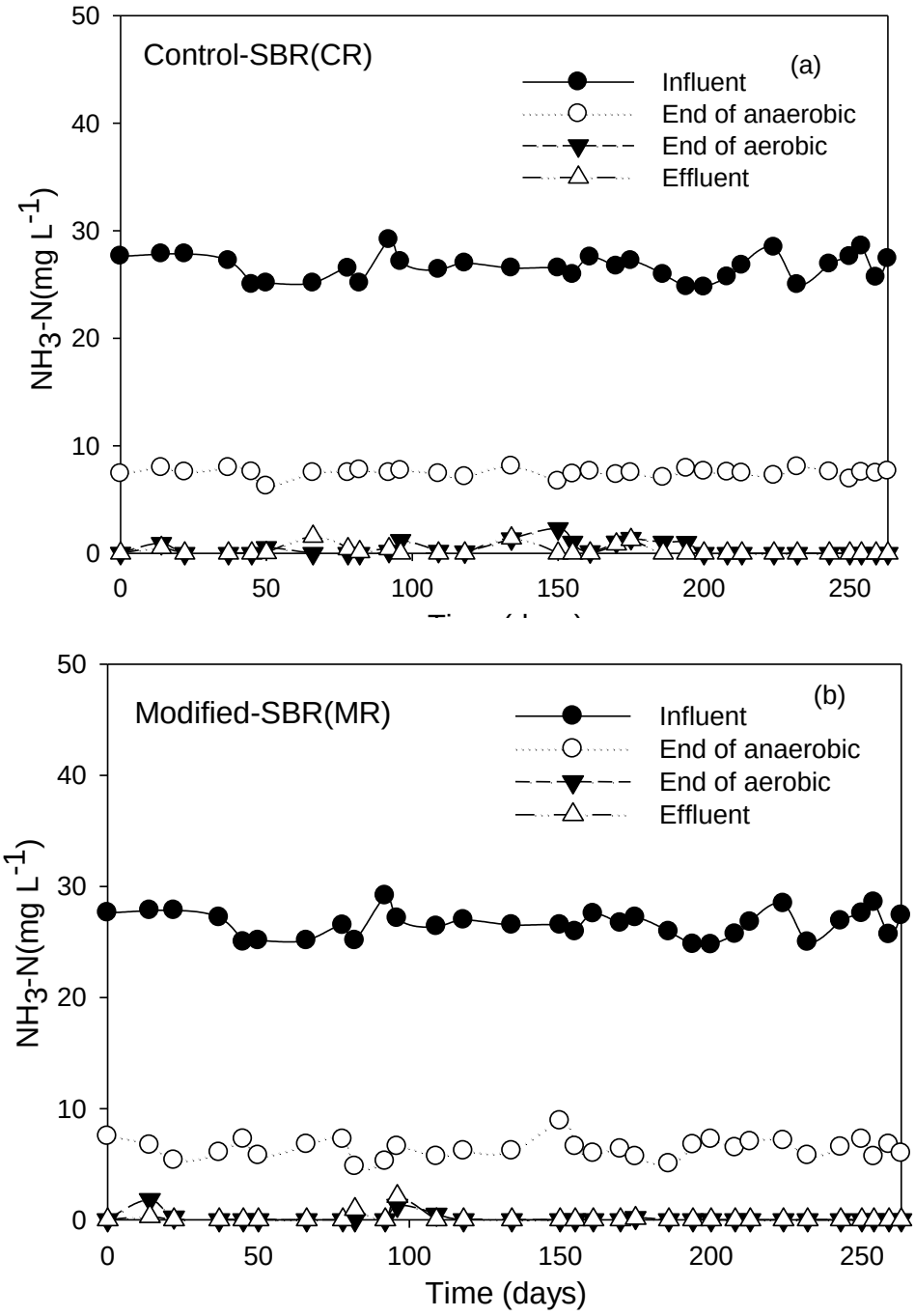


Figure 12: Ammonia nitrogen concentrations profiles for (a) control and (b) modified SBR

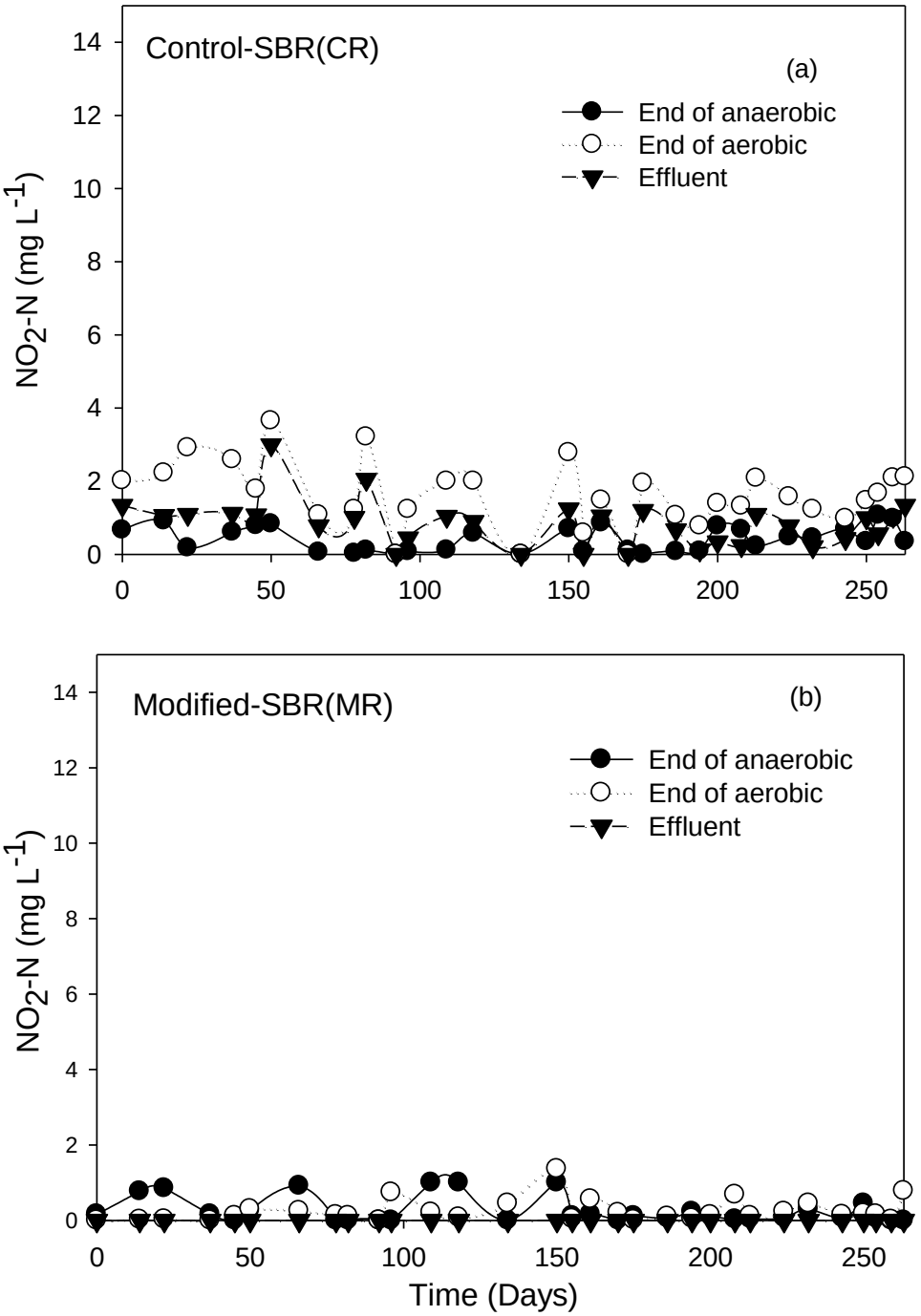


Figure 13: Nitrite nitrogen concentrations profiles in (a) control and (b) modified SBR

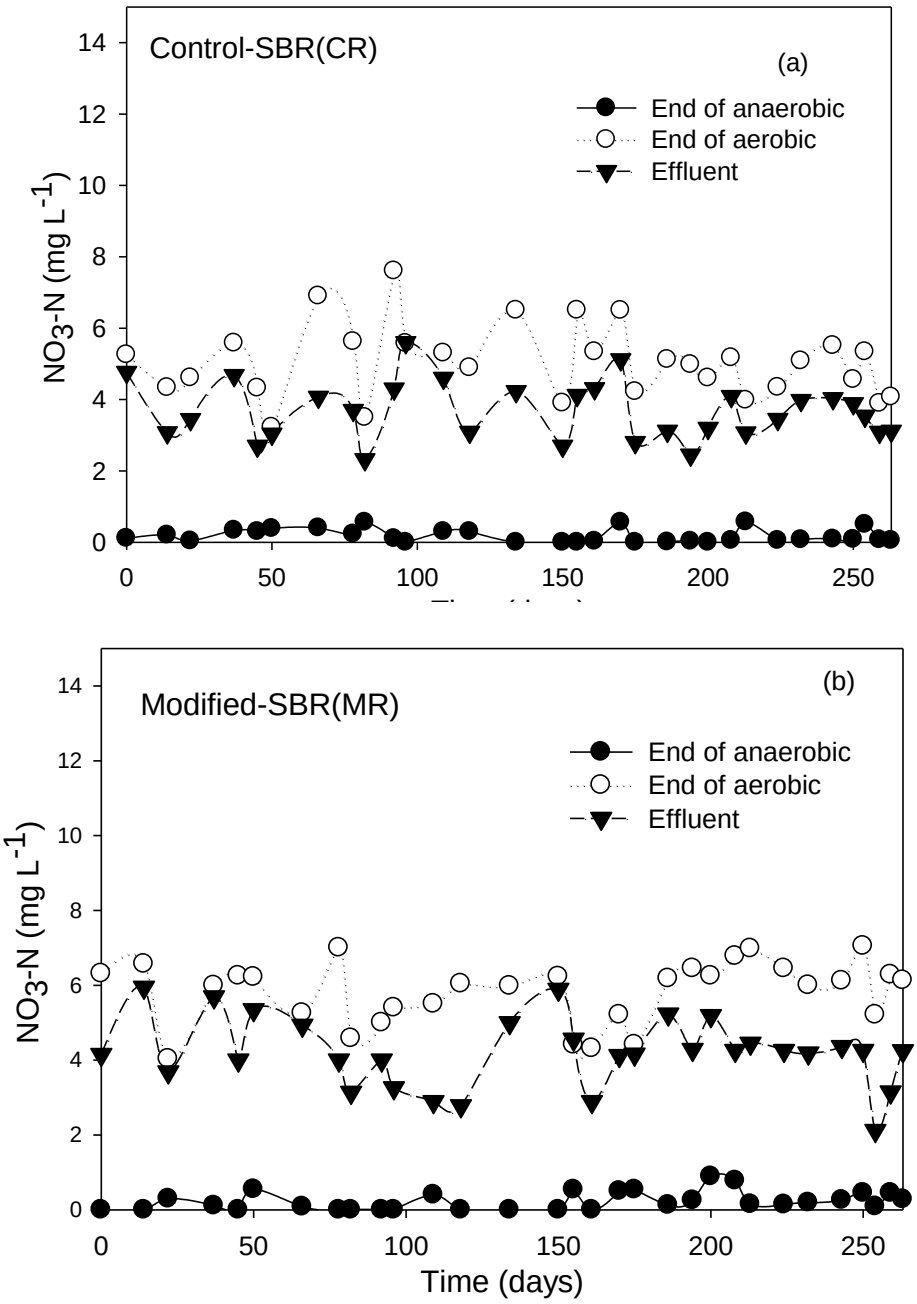


Figure 14: Nitrate nitrogen concentrations profiles in (a) control and (b) modified SBR

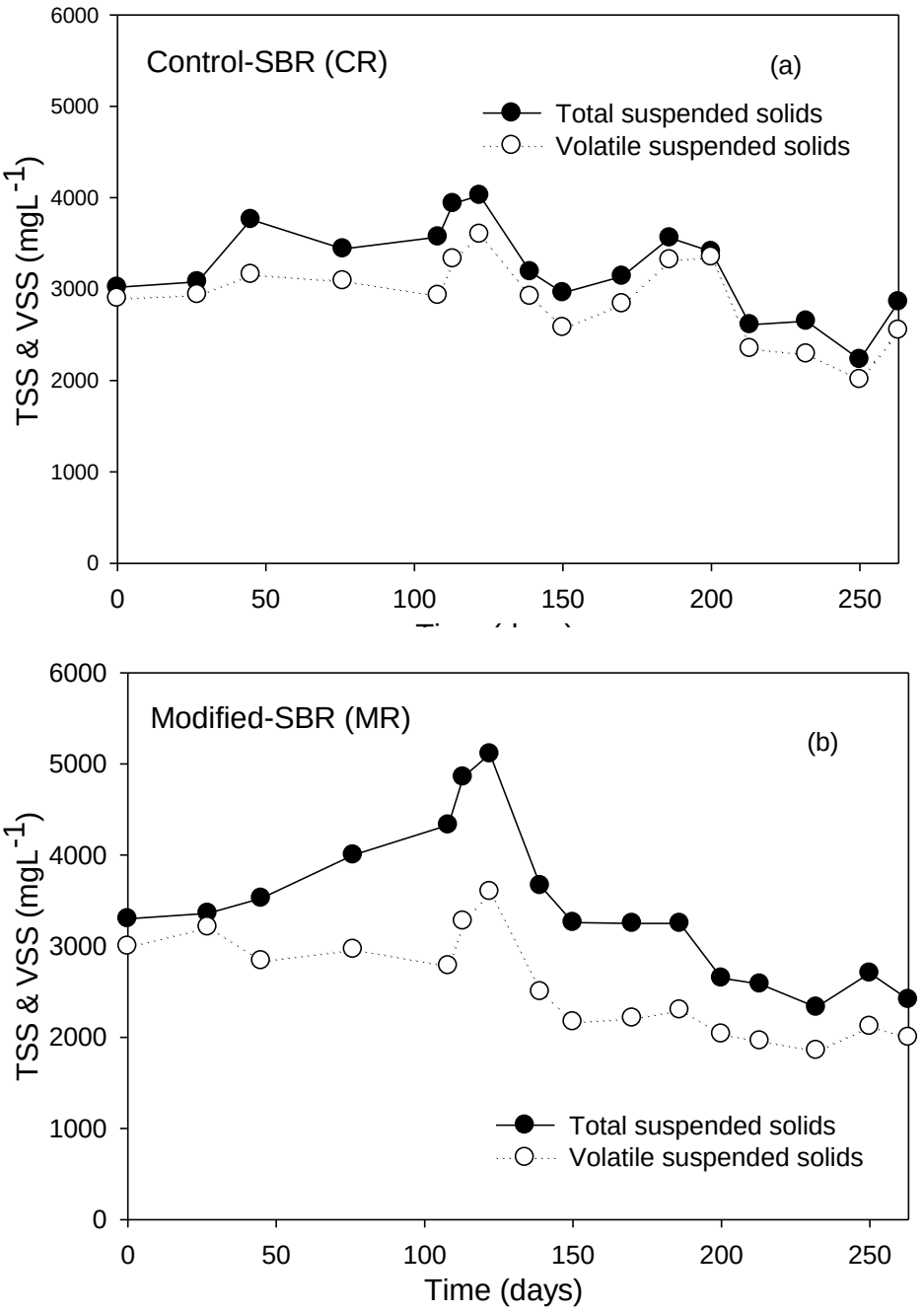


Figure 15: Total and volatile solids in (a) control and (b) modified SBR

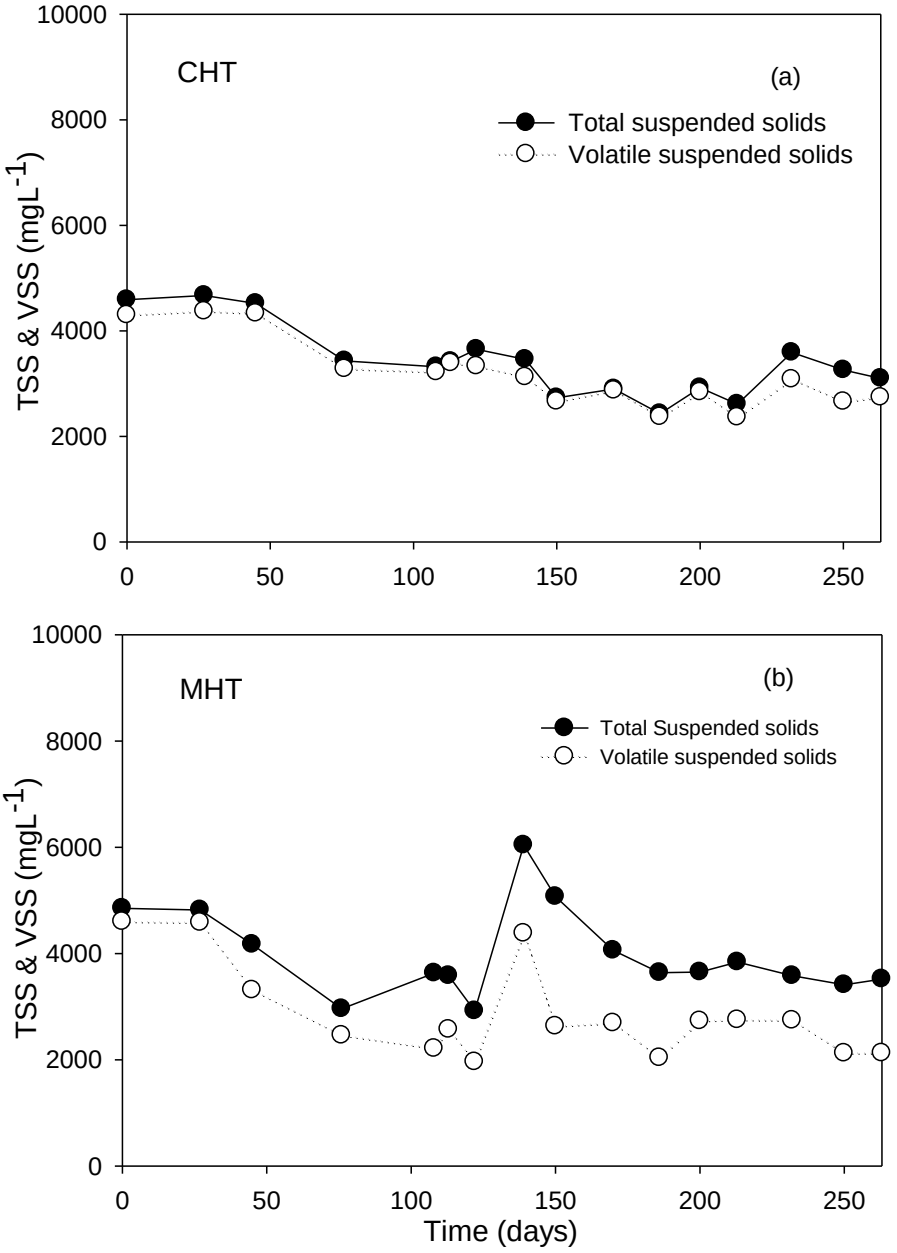


Figure 16: Total and volatile solids in the solids holding tank attached to the control (a) and modified SBR (b)

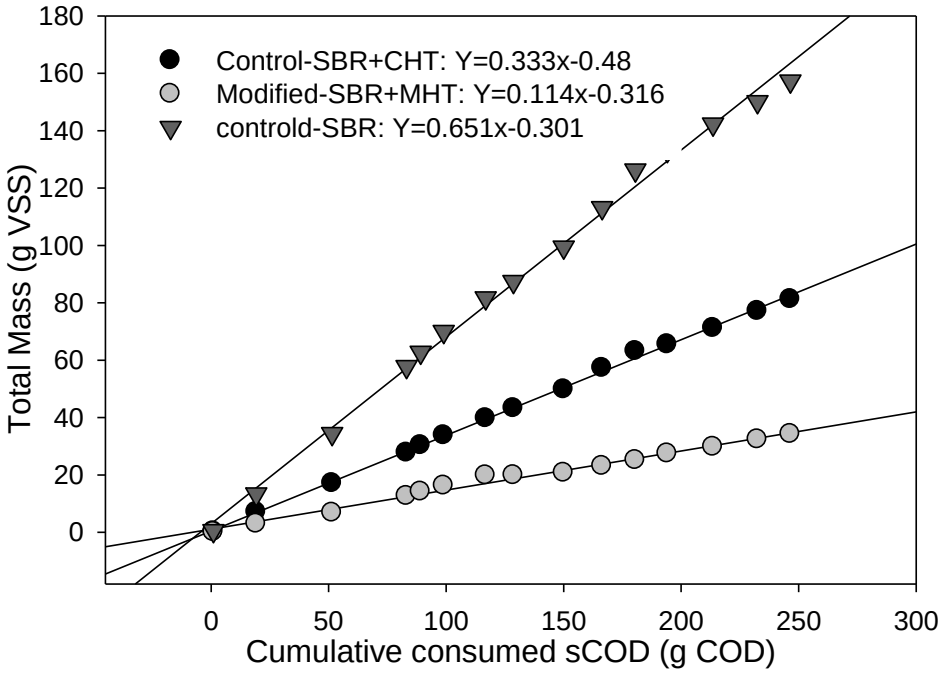


Figure 17: The overall yield in the control system, modified system and control SBR

Table 2: Results of specific oxygen uptake rate (SOUR)

	SOUR (spiked with Feed A)	**SOUR (spiked with Feed B)**
CR	9.71 mg O_2/(gVSS h)	28.68 mg O_2/(gVSS h)
MR	16.51 mgO_2/(gVSS h)	39.80 mg O_2/(gVSS h)

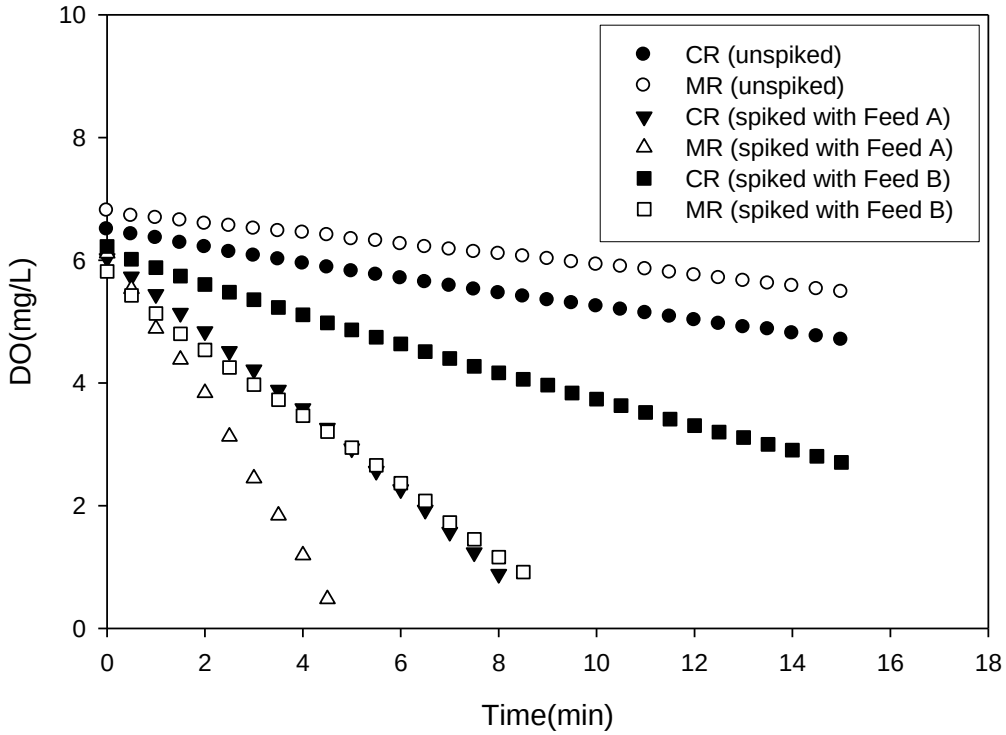


Figure 18: Results of oxygen uptake tests

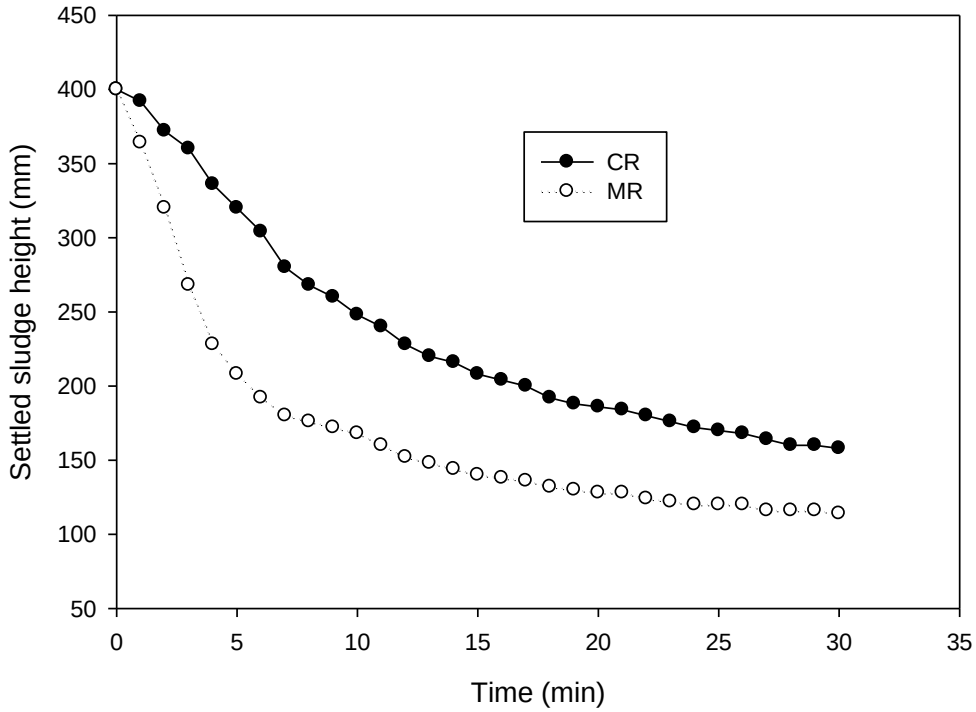


Figure 19: The settling velocity test in both reactors

Table 3: CO_2 formation in serum bottle with C12 substrate with mixed liquors from SBRs

Time (h)	Sample Name	total CO2 N (mol)-average	CO_2 Produced (4h-0h) (mol)	Specific CO_2 produced (4h-0h)/VSS (mol CO_2/g VSS)	MR/CR
0	CR-0h-acid	8.9482E-06 ± 3.74779E-07			
4	CR-4h-acid	7.65689E-05 ± 3.6857E-06	6.76207E-05 ± 4.03361E-06	1.42E-03 ± 8.47E-05	1
0	MR-0h-acid	8.7896E-05 ± 1.52888E-06			
4	MR-4h-acid	1.5639E-04 ± 2.05514E-06	6.84973E-05 ± 9.70266E-07	2.05E-03 ± 2.90E-05	144 %

Table 4: CO_2 formation in serum bottle with C12 substrate with mixed liquors from SBRs

Time (h)	Sample Name	total CO2 N (mol)-average	CO_2 Produced (4h-0h) (mol)	Specific CO_2 produced (4h-0h)/VSS (mol CO_2/g VSS)	MR/CR
0	CR-0h-acid	3.44645E-05 ± 1.0948E-06			
4	CR-4h-acid	9.00041E-05 ± 9.97805E-07	5.55396E-05 ± 1.06016E-06	1.22E-03 ± 2.32E-05	1
0	MR-0h-acid	3.22092E-05 ± 8.23576E-08			
4	MR-4h-acid	9.32616E-05 ± 2.21371E-06	6.10524E-05 ± 2.17255E-06	1.77E-03 ± 6.32E-05	145 %

Table 5: Data for C13 based carbon mass balance: partitioning between gas phase and biomass

Sample Name	total CO2 N (mol)-average	AT % $^{13}C/^{12}C$ [%]	13C (mol)	^{13}C in headspace/spiked ^{13}C ACETATE
CR-0h-acid	1.01E-04	2.043	2.06E-06	0.8%
MR-0h-acid	9.16E-05	4.95	4.53E-06	1.8%

sample name	AT % $^{13}C/^{12}C$ [%]	CO_2 conc. (mol/L)	^{12}C conc. (mol/L)	^{13}C conc. (mol/L)	^{13}C in headspace/spiked ^{13}C ACETATE
Standard	1.122768				
Standard	1.124272				
CR-4h-1	4.885536	0.002932407	0.002789143	0.000143264	56.6%
CR-4h-2	4.880971	0.002944108	0.002800407	0.000143701	56.8%
MR-4h-1	6.739486	0.002834699	0.002643655	0.000191044	75.5%
MR-4h-2	6.751233	0.002849326	0.002656961	0.000192365	76.0%

sample name	biomass(g)	AT % $^{13}C/^{12}C$ [%]	C/biomass	^{13}C in biomass-^{13}C background (mol)	(^{13}C in biomass-^{13}C background)/spiked ^{13}C ACETATE
CR4h	0.0508	1.4604	32.54%	5.25405E-06	41.54%
MR4h	0.0346	1.483	32.00%	3.7368E-06	29.54%
CR Blank		1.078			
MR Blank		1.077			

Table 6: The result of C13 carbon mass balance in the solids holding tank attached to the control and modified reactors

	headspace			
sample	ΔCO_2 (mol)	$\Delta C12\text{-}CO_2$ (mol)	$\Delta C13\text{-}CO_2$ (mol)	specific (mol$C13\text{-}CO_2$/g VSS)
CHT-6 hr	2.78E-05	2.64E-05	1.46E-06	5.01E-05
CHT-24 hr	2.04E-05	1.80E-05	2.45E-06	8.42E-05
MHT-6 hr	2.64E-06	3.01E-06	1.14E-06	4.69E-05
MHT-24 hr	7.32E-06	9.00E-06	2.82E-06	1.16E-04

Table 7: C13 partitioning into biomass for aerated bioreactor samples

	Biomass, in Moles					
	Blank			C13 Spiked		
	0 h	6 h	Dec	0 h	6 h	Incr
Peru	5.02E-05	4.35E-05	6.70E-06	5.14E-05	4.77E-05	-3.70E-06
Albany	5.90E-05	5.64E-05	2.60E-06	6.03E-05	6.04E-05	1.00E-07
Lebanon	3.81E-05	3.65E-05	1.60E-06	4.31E-05	4.39E-05	8.00E-07
Emporia	4.88E-05	4.90E-05	-2.00E-07	5.08E-05	5.37E-05	2.90E-06
Morongo	2.07E-05	1.82E-05	2.50E-06	2.34E-05	2.33E-05	-1.00E-07
Clovis	5.84E-05	5.34E-05	5.00E-06	5.97E-05	5.80E-05	-1.70E-06
Murray	5.50E-05	5.01E-05	4.90E-06	5.72E-05	5.26E-05	-4.60E-06
ID 2963	5.27E-05	5.18E-05	9.00E-07	5.49E-05	5.85E-05	3.60E-06
New Miami	2.21E-05	2.17E-05	4.00E-07	2.55E-05	2.62E-05	7.00E-07
Big Bear	5.96E-05	5.44E-05	5.20E-06	5.51E-05	6.11E-05	6.00E-06

Table 8: Head space CO_2 concentrations for aerated bioreactor

	Head Space, in Moles					
	Blank			C13 Spiked		
	0 h	6 h	Diff	0 h	6 h	Diff
Peru	4.14E-06	5.60E-06	1.46E-06	4.94E-06	6.55E-06	1.61E-06
Albany	8.84E-07	1.99E-06	1.11E-06	1.24E-06	3.24E-06	2.00E-06
Lebanon	8.04E-07	2.02E-06	1.22E-06	1.18E-06	3.48E-06	2.30E-06
Emporia	1.63E-06	1.87E-06	2.40E-07	1.79E-06	3.08E-06	1.29E-06
Morongo	8.43E-07	1.07E-06	2.27E-07	1.24E-06	2.25E-06	1.01E-06
Clovis	7.08E-07	9.51E-07	2.43E-07	1.18E-06	1.42E-06	2.40E-07
Murray	4.15E-07	8.01E-07	3.86E-07	1.02E-06	2.37E-06	1.35E-06
ID 2963	7.71E-07	1.12E-06	3.49E-07	1.21E-06	2.26E-06	1.05E-06
New Miami	1.40E-06	1.38E-06	-2.00E-08	1.73E-06	1.87E-06	1.40E-07
Big Bear	1.83E-06	2.25E-06	4.20E-07	2.54E-06	4.35E-06	1.81E-06

Table 9: Head space CO_2 concentrations for sidestream samples

	Head Space, in Moles					
	Blank			C13 Spiked		
	0 h	6 h	Diff	0 h	6 h	Diff
Peru	3.79E-06	3.89E-06	1.00E-07	4.19E-06	4.45E-06	2.60E-07
Albany	1.71E-06	2.22E-06	5.10E-07	2.19E-06	2.38E-06	1.90E-07
Lebanon	2.83E-06	2.49E-06	-3.40E-07	2.27E-06	3.76E-06	1.49E-06
Emporia	2.90E-06	1.87E-06	-1.03E-06	1.79E-06	3.08E-06	1.29E-06
Morongo	2.30E-06	2.49E-06	1.90E-07	2.51E-06	3.06E-06	5.50E-07
Clovis	1.81E-06	2.22E-06	4.10E-07	2.03E-06	2.69E-06	6.60E-07

Table 10: C13 partitioning into biomass for sidestream samples

	Biomass, in Moles					
	Blank			C13 Spiked		
	0 h	6 h	Dec	0 h	6 h	Incr
Peru	9.30E-05	1.04E-04	-1.10E-05	1.06E-04	1.07E-04	1.00E-06
Albany	7.41E-05	6.66E-05	7.50E-06	7.03E-05	6.37E-05	-6.60E-06
Lebanon	8.28E-05	8.61E-05	-3.30E-06	8.43E-05	8.97E-05	5.40E-06
Emporia	7.44E-05	6.77E-05	6.70E-06	7.52E-05	7.25E-05	-2.70E-06
Morongo	8.81E-05	8.38E-05	4.30E-06	8.76E-05	8.83E-05	7.00E-07
Clovis	5.67E-05	5.52E-05	1.50E-06	5.83E-05	5.97E-05	1.40E-06

CHAPTER 5

CONCLUSIONS

From this study, several conclusions can be derived:

- The lab scale control SBR, which was maintained 10 days SRT5, and modified SBR, which was operated at sufficiently high SRT, reached steady-state performance and achieved an average PO_4^{3-}-P removal of 85%, NH_3-N removal of 99%, and 100% COD removal. The modified SBR performed slightly better than control SBR on NH_3-N and PO_4^{3-} removals.
- The oxygen uptake rate of the biomass from both reactors showed that heterotrophic and nitrification activity in modified SBR was higher than those in control SBR.
- The solids yield in the modified reactor was 60% less than in the control system without any negative effect on the effluent and the settling quality. It can also be concluded that the sludge reduction mechanism in the modified system is a combination of both mainstream and sidestream reactors.
- The mechanism for the solids loss appear to result from the carbon mass balance because of maintenance and endogenous metabolism in the main reactor, also, the solubilization of organic matter in the sidestream reactor is degraded when organic matter is returned back to the main reactor.
- For the full scale Cannibal system, successfully operating carbon mass balance tests tend to have a greater specific CO2 production in the better performance plants than the poorer performing plants as measured the headspace.

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